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The use of statins potentiates the insulin sensitizing effect of exercise training in obese males with and without type 2 diabetes.

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Abstract

Exercise-training is advocated in insulin resistance and statins are used to treat hyperlipidemia, two cardiometabolic risk-factors often presenting concurrently. Statin intake may blunt mitochondrial function and the adaptive response to exercise training. Thus, combining exercise training with statins administration may have adverse effects. We examined if improvements in cardiometabolic risk-factors, insulin sensitivity and mitochondrial function mediated by progressive exercise training are affected by statin use.

Methods

Fourteen obese elderly males on statins (ST) and 22 matched control subjects (C) were examined. Results on *in vivo* mitochondrial function (MRS), mitochondrial density (western blot), insulin sensitivity (clamp) and metabolic flexibility (indirect calorimetry) were compared before and after the 12 week combined progressive training program (3x/week, 45 minutes/session).

Results

Except for LDL cholesterol, all pre-training values were comparable between statin users and control subjects. *In vivo* mitochondrial function (MRS) and mitochondrial density (western blot) improved by training in both groups. Interestingly, blood-lipid profile, insulin sensitivity (+72%) (clamp), non-oxidative and oxidative glucose disposal (+38% and +112%) and insulin-mediated suppression of fat oxidation (-62%) (indirect calorimetry) improved only in ST.

Conclusion

Statin treatment did not impede exercise performance or tolerance, mitochondrial function or mass. In addition, training-induced improvements in glucose homeostasis were preserved in ST. Strikingly, the insulin sensitizing effect of training was more prominent in ST than in C. The combined prescription of statins along with exercise training is safe and should be considered for subjects prone to develop insulin resistance.

Introduction

Hyperlipidemia and hyperglycemia are frequently reported in obese subjects and are highly associated with the development of cardiovascular disorders, the primary cause of death in obesity. Whereas treatment with HMG-CoA reductase inhibitors like statins is often prescribed in case of hyperlipidemia [1, 2], implementing routine physical exercise in daily life is advocated in treatment of hyperglycemia [3]. Hyperlipidemia and hyperglycaemia, however, often present concurrently. Hence, combining the current guidelines would imply combining statin treatment with physical exercise. Recently released guidelines from the American Diabetes Association state that in diabetic individuals >40 years of age, statin use should be added to lifestyle therapy in order to reach target levels of LDL cholesterol < 2.6 mmol/l in patients with type 2 diabetes (T2D) or < 1.80 mmol/l in T2D with established CVD [4]. The use of statins, however, has been associated with myotoxicity, mitochondrial dysfunction [5-9] and muscular damage [7, 10]. In addition, it has been suggested that statin use may blunt the adaptive response to exercise training [11, 12].

To examine the role of statins in exercise training-mediated improvements in cardiometabolic risk-factors, markers of *in vivo* mitochondrial function and insulin sensitivity, we here evaluate these effects after 12 weeks of training in a group of elderly male obese healthy and T2D subjects, stratified to the use of statins.

Research design and methods

Subject recruitment and stratification

Initially, we recruited 38 sedentary elderly male obese subjects of which 18 subjects had been diagnosed with T2D for at least one year [13]. Diabetic patients had well-controlled diabetes (HbA1c = \pm 7.2%) and were using oral anti-diabetic agents (metformin only, or in combination with SU derivatives). The other 20 subjects were categorized as normoglycemic. Glycemic control was checked for by measuring HbA1c, and glucose tolerance by performing an oral glucose tolerance test. Subjects were informed about the nature and risks of the experimental procedures before their written informed consent was obtained. The study has been carried out in accordance with the Declaration of Helsinki (2000) of the World Medical Association and was approved by the institutional medical ethical committee.

Retrospectively, the entire group of 38 subjects was stratified according to the use of statins. Two subjects on fibrates were excluded from analysis. Statin use was defined as using any type of statins, for over a year at the onset of the study, irrespective of dosage. Individual data on statin use can be found in the supplementary material. Stratification resulted in 14 subjects on statins (ST) and 22 subjects without statin treatment (C) with 8 T2D subjects in both groups. None of the baseline characteristics was significantly different between the C and ST group, except for LDL cholesterol which was lower in the patients using statins (subject characteristics see table 1). To examine if the type of statin and the dosage used may have affected the outcome of the present study, we ranked the statins on their reported LDL lowering potency, taking into account the dosage used. Using this marker of potency as an independent variable we performed correlative analysis with all parameters under investigation.

Exercise training protocol

All subjects were engaged in an exercise program for 12 weeks, consisting of a combination of aerobic and resistance exercise. Subjects were monitored extensively during the training period and attention has been paid to follow up conversations with respect to their motivation. Aerobic exercise was carried out on a cycling ergometer twice a week for 30 minutes at 55% of their previously determined maximal work load. Training sessions took place in small groups of 3 to 4 persons and as a measure of exercise intensity, heart rate was monitored and registered in a

training diary every 5 minutes. Maximal workload was re-evaluated after 6 weeks of training and training load was adjusted accordingly.

Resistance exercise was performed once a week for 40 minutes. The training involved a “circuit” of eight exercises, focussing on large muscle groups (i.e., chest press, leg extension, lat pull down, leg press, triceps curl, biceps curl, crunches and horizontal row). A first series of 8 repetitions was performed at 55% of their pre-determined maximal voluntary contraction (MVC), followed by 2 series of 8 repetitions at 75% MVC [13]. Resistance training was given individually and MVC was re-assessed every 4 weeks.

Hyperinsulinemic-euglycemic clamp

Dietary habits were stable, physical exercise was avoided the 2 days prior to the clamp procedure and anti-diabetic medication was withdrawn 7 days prior to the clamp. The use of statins was continued throughout the study. Insulin sensitivity was measured by a 3-hour hyperinsulinemic-euglycemic clamp, before and after the training period. A primed constant infusion of glucose tracer ([6,6-2H₂]glucose) was initiated at t=0 minutes to determine non-insulin stimulated rates of glucose appearance (*R_a*) and disposal (*R_d*). At t=180, the actual clamp procedure was started with a primed constant infusion of insulin (40mU/m²/min) [13]. Endogenous glucose production (EGP) was calculated as *R_a* minus exogenous glucose infusion rate. Non-oxidative glucose disposal was calculated as *R_d* minus carbohydrate oxidation. In the non-insulin stimulated period (t=150-180) and under steady clamp conditions (t=330-360), blood samples and indirect calorimetry measurements (ventilated hood) were obtained. A needle muscle biopsy was obtained from the m. vastus lateralis before starting the tracer infusion.

Blood sample analysis

Blood was sampled and analysed as described previously [13]. Concentrations of total cholesterol (ABX Diagnostics, Montpellier, France), HDL cholesterol (precipitation method; Roche Diagnostics Corporation, Indianapolis, IN), and triglycerides corrected for free glycerol (Sigma-Aldrich Chemie, Steinheim, Germany) were analysed enzymatically. Serum LDL cholesterol concentrations were calculated by using the formula of Friedewald. High sensitive C-reactive protein (hsCRP) was measured on Cobas Mira with a commercially available kit (Kamiya Biomedical Company, Seattle, WA, USA).

Metabolic flexibility

Metabolic flexibility is the ability to switch from predominantly fat oxidation in the fasted state to glucose oxidation under insulin stimulated conditions [14]. Metabolic flexibility was expressed as the change in respiratory exchange ratio (RER) from the fasted state to the insulin-stimulated condition. Substrate oxidation was calculated according to Frayn with protein oxidation considered negligible (carbohydrate oxidation (umol/min/kg) = (4,585 * VCO₂ – 3,226 * VO₂) / BW / 180 * 1000 and lipid oxidation rates (umol/min/kg) = (1,695* VO₂ – 1,701 * VCO₂) / BW / 860 * 1000) [15].

MRS-based measurement of mitochondrial function

Maximal muscle strength during one-legged knee-extension exercise was assessed a few days prior to the actual ³¹P-MRS measurement with an incremental protocol in an MRS compatible exercise device. During this test subjects were asked to perform knee-extensions at 0.5 Hz, while increasing the load to be lifted every 30 seconds by 500 g, with an initial weight of 5 kg. The test was performed until exhaustion or until the frequency of 0.5 Hz could no longer be maintained (maximal load). The phosphocreatine (PCr) lowering exercise session during the test-day was performed at 60% of this pre-determined load.

Baseline ^{31}P -MRS measurements were performed on a 1.5 T whole body scanner (Intera, Philips Health Care, Best, the Netherlands). A 6 cm surface coil was used for localization and was fixed in the middle of the vastus lateralis muscle. A series of partially saturated spectra (free induction decays) was acquired (TR = 4 seconds, 1 measurement, spectral bandwidth 1500 Hz, adiabatic pulse). Knee extension exercise was performed at 0.5 Hz to an acoustic cue on a home-built MR compatible ergometer with a pulley system. The PCr lowering exercise was performed with identical weights before and after training.

The acquisition time during time series was structured as follows: 40 sec of rest, 5 minutes of knee-extension exercise and 5 minutes of recovery. After application of phase correction, line-broadening and DC correction, spectra were fitted in the time domain with the AMARES algorithm [16] in the jMRUI software (<http://www.mrui.uab.es>) [17] using prior knowledge. Five peaks were fitted with gaussian curves (Pi, PCr, and 3 ATP peaks) in the partially saturated spectra of the time-series. To determine the pH during the exercise period, five consecutive spectra of the time-series were added and the pH was calculated in jMRUI from the frequency shift between PCr and Pi peak. The time-course of the PCr amplitude (PCr(t)) during the last 20 seconds of exercise (steady state) and during the recovery period was fitted with MATLAB software (The Mathworks. Inc.), as described earlier [18], assuming a monoexponential PCr recovery. Post-exercise PCr resynthesis rate is an almost exclusively oxidative process and is hence a good reflection of mitochondrial oxidative function. The rate constant of the monoexponential recovery is given. The higher the rate constant (s^{-1}), the better *in vivo* mitochondrial function.

Mitochondrial density

Five different structural components of the electron transport chain were measured at the protein level as a reflection of mitochondrial density [13]. The ND6 subunit of complex I, the 30-kDa Ip subunit of complex II, the 47-kDa core protein 2 of complex III, subunit II of cytochrome *c* oxidase (COXII), and the subunit of the F1F0 ATP synthase (complex V) were measured using a monoclonal antibody cocktail of 5 monoclonal antibodies directed to the subunits mentioned (MitoSciences, Eugene, OR) as previously described [19]. As these subunits differ considerably in molecular mass, this antibody gives 5 distinct bands in human samples. Each individual band reflects one of the mitochondrial subunits. In short, muscle biopsies were homogenized in ice-cold Tris-EDTA buffer at pH 7.4, and then the homogenates were sonicated for 15 sec. Subsequently, two volumes of each skeletal muscle homogenate and one volume of SDS-sample buffer were boiled for 4 min. Next, 13% polyacrylamide gels containing 0.1% SDS were loaded with equal amounts of protein from each sample, and electrophoresis was performed using a Mini-Protean 3 Electrophoresis Cell (Bio-Rad Laboratories, Hercules, CA). After gel electrophoresis, the gel was scanned and the optical density of all individual bands (per subject per subunit) was measured. To adjust for inter-gel variation, the optical density of the band of interest per subject was normalized to the mean optical density of the complete gel. Protein content is expressed as arbitrary units (AU).

Statistics

Statistical analyses were performed two-sided using SPSS for Windows 15.0 software (SPSS, Chicago, IL). Statistical significance was set at $p < 0.05$. A two-way ANOVA model for repeated measures was applied using control and statin users as between subject variables and pre- and post training data as repeated within subject variables. Differential responses between statin users and non statin users were examined using the interaction terms. Also, in case of a significant training effect, differential responses of T2D and healthy subjects within statin users and non statin users were examined.

Results

Subjects

Before training, body weight (BW) (94.9 ± 2.8 and 93.2 ± 2.9 kg), BMI (30.1 ± 0.8 and 30.0 ± 0.9 kg/m²), and age (59.2 ± 1.0 and 58.4 ± 0.9 years) were comparable in C and ST respectively. Training did not alter body weight and BMI in C while body weight but not BMI tended to decline in ST (from 93.2 ± 3.2 to 91.9 ± 3.1 kg, $p=0.09$ and from 30.0 ± 0.9 to 29.6 ± 0.8 kg/m²). Fat mass declined in C (from 31.2 ± 1.8 to 30.4 ± 1.8 kg after training, $p=0.02$) whereas there was a tendency towards a decreased fat mass in ST (from 27.9 ± 2.3 to 26.4 ± 2.2 kg after training; $p=0.10$). Fat free mass was similar between both groups and was unaffected by training.

Before training, maximal oxygen uptake (VO₂max/kg BW) and maximal workload was comparable in both groups (VO₂max/kgBW 28.6 ± 1.0 ml/kg and 27.7 ± 1.5 ml/kg in C and ST respectively, $p=0.63$; workload 208 ± 9 Watt 201 ± 10 Watt in C and ST respectively, $p=0.61$). Exercise training improved VO₂max/kg body weight and maximal workload significantly and to the same extent in C and ST (VO₂max $+7.0 \pm 2.2\%$ and $+11.7 \pm 3.2\%$ respectively, $p<0.01$; workload $+14.6 \pm 2.7\%$ and $+16.1 \pm 2.3\%$ in C and ST respectively, $p<0.01$). Muscle strength was comparable before training (83.5 ± 3.2 kg and 87.4 ± 4.0 kg in C and ST respectively, $p=0.46$) and improved significantly and to the same extent in both groups ($+24.7 \pm 1.9\%$ and $+21.6 \pm 2.1\%$ in C and ST respectively, $p<0.01$). In the rare occasion of missing a training session, this session was rescheduled within a week of the original training date. As maximally achieved workload during the maximal test was similar in both groups and the number of training sessions performed was identical, total workload performed during the training sessions across groups was comparable.

Glucose and insulin profile

Fasting glucose levels (7.2 ± 0.4 mmol/l and 7.5 ± 0.5 mmol/l in C and ST) and HbA1c levels ($6.4 \pm 0.2\%$ and $6.6 \pm 0.3\%$ in C and ST) were similar at the onset of the training program. Twelve week of exercise training did not change HbA1c or fasting glucose levels in either group. Fasting plasma insulin levels did not differ significantly before onset of the training program between C and ST (17.2 ± 2.0 vs. 17.9 ± 2.0 mU/l in C and ST) but decreased significantly in both groups after training (from 17.2 ± 2.0 mU/l to 15.7 ± 1.7 mU/l in C and from 17.9 ± 2.0 mU/l to 15.0 ± 1.7 mU/l in ST, $p<0.05$ in both groups).

Lipid profile

Pre-training, insulin-mediated suppression of lipolysis, measured as the drop in plasma FFA upon insulin infusion, was comparable between groups. Training did not affect insulin-mediated suppression of lipolysis in C (suppression pre-training $81.7 \pm 1.6\%$ vs $83.0 \pm 1.5\%$ post-training), but improved significantly in ST (from $77.7 \pm 1.8\%$ to $82.9 \pm 1.7\%$, $p=0.01$).

Triglycerides were comparable between groups and were unaffected by training. Also total cholesterol before training was similar in C and ST. Training reduced total cholesterol significantly in ST (from 5.0 ± 0.2 to 4.6 ± 0.2 mmol/l, $p=0.03$), but not in C (from 5.4 ± 0.2 to 5.2 ± 0.1 mmol/l), resulting in a tendency towards lower total cholesterol values in ST compared to C ($p=0.07$). LDL cholesterol was significantly higher in C compared to ST pre-training (3.5 ± 0.1 mmol/l vs 2.9 ± 0.2 mmol/l in C and ST, $p=0.05$) and tended to decline in both groups (from 3.5 ± 0.1 to 3.3 ± 0.2 mmol/l in C, $p=0.07$ and from 2.9 ± 0.2 to 2.7 ± 0.2 mmol/l in ST, $p=0.07$). HDL cholesterol and hsCRP was comparable in C and ST and was unaffected by training (Table 1).

Whole-body insulin-stimulated glucose uptake

Insulin-stimulated glucose uptake, as determined by the delta glucose disposal rate during the hyperinsulinemic euglycemic clamp (delta Rd), was not different between C and ST pre-training (12.7 ± 2.1 and 9.5 ± 2.5 umol/kg/min in C and ST, $p=0.33$). C and ST responded differentially to training as indicated by significant interaction ($p<0.01$). Delta Rd did not change after training in

C (from 12.7 ± 2.1 to 13.6 ± 1.9 $\mu\text{mol/kg/min}$), but improved significantly in ST (from 9.5 ± 2.5 to 16.3 ± 2.3 $\mu\text{mol/kg/min}$, $p < 0.01$). Non-oxidative glucose disposal (from 4.7 ± 2.1 to 10.0 ± 2.1 $\mu\text{mol/kg/min}$, $p < 0.01$) as well as oxidative glucose disposal (from 4.7 ± 0.9 to 6.5 ± 0.7 $\mu\text{mol/kg/min}$, $p < 0.01$) improved significantly in ST upon training, but did not change in C (table 2, with a significant interaction effect between groups for NOGD; $p < 0.01$). Insulin mediated suppression of endogenous glucose production (delta EGP) was comparable pre-training (-6.8 ± 0.8 and -7.1 ± 1.5 $\mu\text{mol/kg/min}$ in C and ST respectively) and was not significantly affected by training, despite a significant interaction effect (Table 2). Data of insulin and glucose disposal is presented in table 2.

Markers of mitochondrial density

Mitochondrial density was evaluated by measuring protein content of 5 structural subunits of the distinct complexes of the electron transport chain. Neither the individual complexes, nor the mean protein content of these complexes revealed a difference in mitochondrial density between C and ST pre-training (0.56 ± 0.10 and 0.71 ± 0.10 AU in C and ST, NS). Training resulted in increased mitochondrial density in both groups after training (0.56 ± 0.10 to 1.29 ± 0.16 AU in C, $p < 0.01$ and 0.71 ± 0.10 to 1.22 ± 0.16 AU in ST, $p = 0.03$) (Figure 1 panel a).

MRS measurement

Pre-training *in vivo* mitochondrial function in C was similar to ST (rate constant: 0.034 ± 0.002 and 0.034 ± 0.003 s^{-1} in C and ST respectively) while after training *in vivo* mitochondrial function improved with 34% and 46% (from 0.034 ± 0.002 to 0.044 ± 0.003 s^{-1} in C, $p < 0.01$ and from 0.034 ± 0.003 to 0.049 ± 0.006 s^{-1} in ST, $p = 0.02$) (Figure 1 panel b).

Metabolic flexibility

Prior to training, metabolic flexibility (ΔRER 0.071 ± 0.008 and 0.062 ± 0.012 , $p = 0.51$), insulin stimulated glucose oxidation (ΔCHOox 4.7 ± 0.7 and 4.7 ± 0.9 $\mu\text{mol/kg/min}$, $p = 0.99$) and suppressed fat oxidation (ΔFATox -0.41 ± 0.05 and -0.34 ± 0.07 $\mu\text{mol/kg/min}$, $p = 0.47$) was comparable between C and ST. Exercise training did not affect metabolic flexibility in C (ΔRER from 0.071 ± 0.008 to 0.083 ± 0.011 , $p = 0.22$) whereas metabolic flexibility improved significantly in ST (ΔRER from 0.062 ± 0.012 to 0.089 ± 0.008 , $p = 0.01$), reflecting a concomitant increase in insulin stimulate glucose oxidation (from 4.7 ± 0.9 to 6.5 ± 0.7 $\mu\text{mol/kg/min}$, $p = 0.01$) (Figure 1 panel c) and suppression of fat oxidation (from -0.34 ± 0.07 to -0.55 ± 0.06 $\mu\text{mol/kg/min}$, $p = 0.01$) (Figure 1 panel d).

Discussion

We examined the effect of training on cardiometabolic risk-factors, markers of *in vivo* mitochondrial function and insulin sensitivity in male obese elderly with and without type 2 diabetes who were on lipid lowering drugs (statins, ST group) and compared these effects with a control group (C) not taking statins.

Maximal exercise capacity and markers of mitochondrial content and function were not negatively impacted by statins. In addition, training-induced improvements in markers of cardiometabolic risk, insulin sensitivity and fuel selection were more prominent, and sometimes even exclusively detectable in ST compared to C. Exercise training mediated improvements in mitochondrial density and function were as prominent in ST as in C. The present study hence shows that exercise prescription on top of statin treatment is safe and at least as beneficial -and for some parameters even more beneficial- for cardiometabolic health, glucose homeostasis and mitochondrial function than exercise training without statins.

We did not find correlations between statin potency and other parameters, suggesting that the findings do not scale with the dosage or potency of the statin used. Furthermore, no interaction

effects were detected between healthy subjects and diabetic subjects, indicating that differences were not specific for healthy or diabetic subjects solely.

Pre-training pleiotropic effects of statins

After stratification to the use of statins, both groups were comparable for all subject characteristics, except for LDL cholesterol (table 1). Also markers for insulin sensitivity, substrate metabolism and mitochondrial function were similar (table 2). Interestingly, this is in contrast with previous studies in rodents [20, 21] and humans, [22] showing improved insulin sensitivity on whole body level and in liver. Another study report a decrease in mtDNA copy number [8], suggesting that statins may affect these parameters somehow. In a more recent study, however, a high dose of statins (80 mg/day simvastatin) did not affect insulin-mediated glucose disposal, hepatic glucose production and myocellular lipid deposition [23].

Exercise tolerance on statins

Classical measures of physical fitness were similar in C as in ST, which is in line with previous reports [24]. A 12-week interval cycling training combined with resistance exercise did not provoke statin-related muscle pain or stiffness and was equally well tolerated in C as in ST. Self-reported muscle-stiffness due to unfamiliar exercise occurred predominantly in the first 1.5 weeks and rapidly disappeared after familiarization to the exercise program. Training-mediated improvements in exercise capacity were similar in both groups. Hence, the data of the present study do not support the hypothesis that the use of statins provokes muscle pathology or may blunt the adaptive responses to exercise training, as suggested previously [25]. However, the present study applied a tailored progressive exercise training program to avoid the development of muscle soreness, whereas other investigations applied eccentric exercise and exercise bouts on maximal intensity [11, 12], types of exercise prone to the development of muscle soreness. Also, none of the ST subjects in the present study reported muscle related complaints under resting conditions after starting their statin treatment. We can hence not exclude that the subjects in the present study represent a statin-tolerating subgroup and that this may contribute to the fact that none of them experienced muscle related problems whilst exercising.

Post-training pleiotropic effects of statins

In contrast to previous reports in humans [6] we did not detect any (negative) effects of statin use on mitochondrial content and function pre-training and showed similar improvements upon training. It should be noted though, that in the present study the dosage of statins used was in general lower [6].

Intriguingly, exercise training in ST improved insulin stimulated glucose disposal, both by an increase in oxidative as well as in non-oxidative glucose disposal, without an effect on hepatic insulin sensitivity. The improvement in non-oxidative glucose disposal is of particular interest, as we previously identified non-oxidative glucose disposal as the non-responsive culprit for training-mediated improvements in type 2 diabetic patients [13]. In contrast, in C, insulin-stimulated non-oxidative glucose disposal did not improve significantly after training.

One of the characteristics of insulin resistant muscle is metabolic inflexibility (delta RER). Here we observed that metabolic flexibility improved upon training in ST, indicating that statin use does not interfere with training-induced metabolic improvements. The improvement in metabolic flexibility was less pronounced (and non-significant) in C.

Putative mechanisms of statin-mediated potentiation of the beneficial effect of exercise training

We strikingly observed that the insulin sensitizing effects of exercise training were potentiated by the use of statins, but the precise mechanism(s) remains to be established. Candidate mechanisms

include a statin mediated reduction in superoxide-induced cell damage [26], statin mediated-reduction of low-grade inflammation [27], or a reduction in FFA acid flux and hepatic fat storage [28] resulting in improved hepatic insulin sensitivity. The present study does not allow conclusions on the putative effects of statins on superoxide production or superoxide mediated cell damage. Using hsCRP as a marker for low-grade inflammation, we report no differences in low-grade inflammation pre-training between C and ST and did not detect any training effects on hsCRP. Recently it has been shown that even a high-dose of simvastatin (80 mg/daily) does not affect hepatic lipid stores or insulin sensitivity significantly [23]. This is in line with the present study showing similar insulin mediated suppression of hepatic glucose output pre-training in C and ST.

Many patients taking statins also are taking other drugs targeting cardiovascular risk control. The most clear design to examine the effects of the use of statins on exercise mediated improvements in glucose homeostasis and cardiovascular risk would be a double blind, placebo controlled, randomized clinical trial. A disadvantage, however, would be that only subjects can be included who were not on statins before the start of the study while in daily life patients usually been taking statins for quite some time even before they are advocated to physical exercise. Hence, we feel that the design of the present study provides a valuable and valid representation of what happens when subjects implement regular exercise in their daily life, on top of any medication they are taking already. The drawback of our design is that the statin group is not homogeneous for some of the output parameters examined. The fact that we indeed do observe significant differences for the key outcome parameters, however, indicates the effects reported are strong enough to reach significance, even with the relatively small group size shown.

In conclusion, the present study shows in elderly males with moderate obesity that statin treatment did not inhibit exercise-induced improvements with respect to mitochondrial density and function. Rather, improvements in total cholesterol, insulin stimulated lipolysis, insulin sensitivity, non-oxidative glucose disposal, metabolic flexibility, and substrate oxidation were more pronounced and/or even exclusively detectable when exercise and statin use was combined. This study suggests that combining physical exercise training in subjects who well-tolerate statin treatment under resting conditions may be at least as beneficial, and for some parameters even more beneficial, than exercise training exclusively. Thus, the present findings indicate that the recently published guidelines of the ADA to prescribe statins to all type 2 diabetic subjects over the age of 40 can be safely combined with previous guidelines on lifestyle interventions.

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Disclosure

Authors have no potential conflicts of interest, relevant to this article.

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Table 1: subject characteristics

Subject characteristics	C		ST	
	Pre-training	Post-training	Pre-training	Post-training
Age (years)	59.2 ± 1.0		58.4 ± 0.9	
Subjects diagnosed with diabetes	8		8	
Weight (kg)	94.9 ± 2.8	94.4 ± 2.9	93.2 ± 3.2	91.9 ± 3.1
Height (cm)	177.6 ± 1.3		177.2 ± 1.5	
BMI (kg/m ²)	30.1 ± 0.8	30.0 ± 0.9	30.0 ± 0.9	29.6 ± 0.8
Body fat (%)	32.4 ± 1.3	31.6 ± 1.3*	29.6 ± 1.8	28.4 ± 1.8
FM (kg)	31.2 ± 1.8	30.4 ± 1.8*	27.9 ± 2.3	26.4 ± 2.2
FFM (kg)	64.5 ± 1.9	64.9 ± 1.9	65.3 ± 2.1	65.5 ± 2.1
VO ₂ max (ml/min/kg)	28.6 ± 1.1	30.5 ± 1.2*	28.5 ± 1.4	31.7 ± 1.7*
Wmax (Watt)	208 ± 9	236 ± 9*	201 ± 10	231 ± 10*
Average strength (kg)	83.5 ± 3.2	102.9 ± 3.7*	87.4 ± 4.0	105.3 ± 4.4*
Fasting glucose (mmol/l)	7.2 ± 0.4	6.9 ± 0.4	7.5 ± 0.5	7.3 ± 0.6
HbA1c (%)	6.4 ± 0.2	6.3 ± 0.2	6.6 ± 0.3	6.7 ± 0.3
Triglycerides (mmol/l)	1.6 ± 0.1	1.6 ± 0.2	1.7 ± 0.2	1.6 ± 0.1
Total Cholesterol (mmol/l)	5.4 ± 0.1	5.2 ± 0.2	5.0 ± 0.2	4.6 ± 0.2*
Low density lipoproteins (mmol/l)	3.5 ± 0.1	3.3 ± 0.2	2.9 ± 0.2#	2.7 ± 0.2#
High density lipoproteins (mmol/l)	1.2 ± 0.0	1.2 ± 0.1	1.3 ± 0.1	1.3 ± 0.1
HsCRP (mg/l)	2.0 ± 0.3	1.8 ± 0.3	1.6 ± 0.5	1.6 ± 0.5

Data are expressed as mean ± SE., #ST significantly different from control group, *post-training significantly different from pre-training,

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Table 2: Substrate kinetics pre- and post training

	C		ST	
	Pre-training	Post training	Pre-training	Post training
Plasma insulin (mU/l)				
Basal	17.2 ± 2.0	15.7 ± 1.7*	17.9 ± 2.0	15.0 ± 1.7*
Clamp	109.4 ± 5.4	107.8 ± 4.2	111.2 ± 4.5	107.9 ± 5.1
Plasma FFA (µmol/l)				
Basal	498.6 ± 18.3	498.7 ± 31.1	473.3 ± 31.0	421.4 ± 27.9
Clamp	90.2 ± 7.7	81.5 ± 8.2	103.9 ± 10.0	71.1 ± 8.3*§
Rd glucose (µmol/kg/min)				
Basal	10.0 ± 0.8	8.7 ± 0.7	10.9 ± 0.9	8.7 ± 0.7*
Clamp	22.7 ± 2.0	22.3 ± 2.1	20.3 ± 2.2	25.0 ± 2.1*§
Delta	12.7 ± 2.1	13.6 ± 1.9	9.5 ± 2.5	16.3 ± 2.3*§
EGP (µmol/kg/min)				
Basal	9.7 ± 0.6	9.3 ± 0.6	9.7 ± 0.7	7.9 ± 0.8
Clamp	2.8 ± 0.4	0.8 ± 0.5*	2.7 ± 1.0	1.8 ± 1.3
Delta	-6.8 ± 0.8	-8.5 ± 0.7	-7.1 ± 1.5	-6.5 ± 1.5§
CHO oxidation (µmol/kg/min)				
Basal	6.8 ± 0.5	7.3 ± 0.5	7.8 ± 0.8	7.0 ± 0.5
Clamp	11.5 ± 0.7	12.9 ± 0.8	12.6 ± 1.1	13.5 ± 0.7
Delta	4.7 ± 0.7	5.2 ± 0.8	4.7 ± 0.9	6.5 ± 0.7*
NOGD (µmol/kg/min)				
Basal	3.3 ± 0.8	1.2 ± 0.6*	3.0 ± 1.0	1.8 ± 1.1
Clamp	11.2 ± 1.5	9.5 ± 1.8	7.8 ± 1.9	11.9 ± 2.1*§
Delta	7.9 ± 1.7	8.4 ± 1.7	4.7 ± 2.1	10.0 ± 2.1*§
Lipid oxidation (µmol/kg/min)				
Basal	1.10 ± 0.06	1.02 ± 0.04 *	1.06 ± 0.05	1.15 ± 0.08
Clamp	0.69 ± 0.04	0.57 ± 0.06*	0.71 ± 0.06	0.60 ± 0.04
Delta	-0.41 ± 0.05	-0.45 ± 0.07	-0.34 ± 0.07	-0.55 ± 0.06*

Data are expressed as mean ± SE., #ST significantly different from control group, *post-training significantly different from pre-training, § significant interaction effect between C and ST.

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Figure legends

Panel 1a: mitochondrial density as determined by the mean of five different structural components of the electron transport chain, measured at the protein level, before (black bars) and after training (white bars). Data are expressed as mean \pm SE., *post-training significantly different from pre-training

Panel 1b: *In vivo* mitochondrial function as determined by post-exercise PCr recovery rate as described previously[18], before (black bars) and after training (white bars). Data are expressed as rate constant (s^{-1}). A higher rate constant corresponds with a high *in vivo* mitochondrial function. Data are expressed as mean \pm SE., *post-training significantly different from pre-training,

Panel 1c and Panel 1d: delta carbohydrate oxidation and delta fat oxidation, measured as the change from the fasted state to the insulin-stimulated state, before (black bars) and after training (white bars). Data are expressed as mean \pm SE., *post-training significantly different from pre-training. § significant interaction effect between control group and statin group.

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